Association of Major Histocompatibility Complex Determinants with the Development of Symptomatic and Asymptomatic Genital Herpes Simplex Virus Type 2 Infections

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The clinical spectrum of herpes simplex virus (HSV) infections, ranging from asymptomatic to frequently distressing outbreaks, suggests that there may be immunologic determinants of disease severity that are associated with human leukocyte antigen (HLA) expression. A controlled, prospective study identified several major histocompatibility complex (MHC) class I and II antigens whose frequencies are associated with HSV-2 infection or with frequent symptomatic genital recurrences. Previous studies were hampered by the inability to serologically identify patients with asymptomatic HSV-2 infection. Clinical evaluation and Western blot assay were used to identify 3 subject cohorts: 1 with no prior HSV infections, 1 with HSV-2 antibodies but no recognized symptoms, and 1 with HSV-2 antibodies and frequent genital recurrences. Statistical comparisons of HLA frequencies among these cohorts showed associations of HLA-B27 and -Cw2 with symptomatic disease. Also, HLA-Cw4 was significantly associated with HSV-2 infection. These associations indicate that immunologic factors linked to the MHC influence the risk of HSV-2 infection and disease expression.

Clinicians have long appreciated the pleiotropic manifestations of genital herpes. Symptomatic herpes simplex virus type 2 (HSV-2) infections present as frequent and painful recurrences in some persons, while others recognize only a single primary outbreak without subsequent recurrences or only rare recurrences [1–3]. Recently, another subset of HSV-2–infected patients has been delineated and well characterized: those with no recognized primary illness or recurrences but with subclinical or totally asymptomatic infection and reactivation [4–6]. Both groups of infected individuals shed virus and transmit it to intimate partners and neonates [7–9].

The development of type-specific serologic assays has now permitted the reliable identification of asymptomatically infected people [10]. The ability to accurately define both the symptomatic and asymptomatic ends of the spectrum of HSV-2 infections provides new opportunities to study host factors that could determine the extent to which people will recognize and suffer herpetic recurrences.

One host mechanism that could contribute to the overall response to and clinical expression of HSV-2 infection is the human major histocompatibility complex (MHC), which comprises the loci of genes, including the human leukocyte antigens (HLAs) [11]. HLA-A, -B, and -Cw genes constitute the family of MHC class I antigens, which present endogenously derived foreign peptides to $CD8^+$ cytotoxic T lymphocytes (CTL). MHC class II antigens include HLA-DR and -DQ, which present exogenously derived foreign peptides to $CD4^+$ T helper lymphocytes.

The extreme polymorphism of MHC molecules, most predominant in their peptide binding clefts, is credited with a portion of individual variability in intrinsic immunity. Individual HLAs and the peptides they present influence the vigor with which a response is mounted and, in some instances, whether that response will be directed against self antigens. Thus, HLA determinants can be, and in many instances have been, associated with disease severity and spectrum [12, 13]. HLA-B27 has perhaps the most infamous of disease associations, being strongly linked to the eventual development of spondyloar-

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thropathies and acute anterior uveitis [14]. The haplotype HLA-A1, -B8, -DR3 is associated with heightened susceptibility to dermatitis herpetiformis and autoimmune hepatitis [15] and, recently, with increased risk for human immunodeficiency virus (HIV) disease [16].

As with other targets of host defenses, innate antiviral immune mechanisms rely on MHC class I and class II peptide presentation, suggesting that allelic polymorphisms contribute to individual variability in antiviral immunity. A number of studies have addressed this question with regard to HSV infections [17–20]; however, difficulty in identifying the appropriate uninfected control participants has until now confounded their interpretation. That is, in the absence of type-specific serologic tests, the failure to recognize and accurately diagnose certain individuals (i.e., those who are infected with one or the other HSV type yet remain asymptomatic) diluted groups of truly uninfected controls by their presence. Moreover, there could be no reliable comparison of HLA frequencies among the important groups of symptomatically and asymptomatically infected persons.

We present here the HLA typing results of 3 carefully defined patient populations representing (1) individuals experiencing six or more symptomatic genital HSV-2 recurrences per year (when untreated), (2) individuals who are truly uninfected (HSV-1– and HSV-2–seronegative), and (3) those who have serologic evidence of HSV-2 infection but no symptomatic disease despite evaluation and counseling as to its usual features. The associations we found extend our current understanding of host responses to and the immunopathogenesis of HSV-2 disease.

Materials and Methods

Study population. Otherwise healthy study participants (n = 146) were selected from larger pools of persons being evaluated at three centers for their suitability for vaccine or antiviral drug studies. Screening for these studies necessitated HSV type-specific serologic testing by Western blot [10]. The results occasionally identified HSV-2–seropositive persons who had no history of symptoms consistent with genital herpes and who, after receiving instructions regarding the usual spectrum of symptoms and signs, still claimed no previous symptoms.

We initially sought 150 subjects: 50 with serologically and clinically confirmed genital HSV-2 infection that was reported to recur six or more times per year prior to suppressive antiviral treatment, 50 HSV-2–seropositive yet asymptomatic individuals; and 50 who were seronegative for both HSV-1 and HSV-2. During enrollment and screening at three study sites, 162 subjects underwent HLA determination. Sixteen study participants, who were Asian, Hispanic, or African-American, were excluded from analysis because there were not sufficient numbers of racially matched control subjects.

HLA results of 146 white subjects were analyzed: 47 were seropositive for HSV-2 with or without HSV-1 and had frequent, clinically documented symptomatic genital recurrences; 44 subjects were seropositive for HSV-2 with or without HSV-1 and had asymptomatic infection; and 55 were seronegative for HSV-1 and HSV-2.

Typing methods. Four unrefrigerated 10-mL tubes with each subject's blood in acid citrate dextrose were shipped overnight to the University of California in Los Angeles for HLA typing. Lymphocytes were isolated, and $CD4^+$ and $CD8^+$ T cells were purified by positive selection, using immunomagnetic beads [21, 22]. Class I (HLA-A, -B, -Cw) typing was done by use of serologic methods [21], and class II (HLA-DQ, -DR) typing was done by use of polymerase chain reaction [23, 24]. All data were tabulated according to clinical and serologic study group for statistical analysis.

Statistical methods. HLA data were analyzed to evaluate a possible association of alleles with HSV-2 infection or symptomatic disease. Gene frequencies were estimated from phenotypic data, under the Hardy-Weinberg law, using the maximum likelihood method [25, 26]. For comparing cases and controls in each of the specific alleles at each locus, individual χ^2 and Fisher's exact tests were calculated [26, 27]. Bonferroni's correction for multiple comparisons was performed for the tests that showed significance [26, 28]. Fisher's exact test was also used for detecting a haplotype association with infection or disease [27]. All reported P values were two-tailed unless stated otherwise.

Results

Characteristics of the study population. Table 1 shows the characteristics of the study population with respect to sex, age, HSV serology, and HSV disease, and HLA group.

Gene frequencies of the 3 cohorts at the MHC class I and class II loci. Tables 2–6 show the gene frequencies of the study participants at the MHC class I loci HLA-A, -B, and -Cw and the class II loci HLA-DR and -DQ. HLA-A2, the most common of the HLA-A loci polymorphisms, was most prevalent in all 3 study cohorts. Greater allelic diversity is seen in the HLA-B loci, with HLA-B35 most frequent in HSV-infected participants and HLA-B7 most frequent in uninfected individ-

Table 1. Clinical and demographic characteristics of the study population according to HSV infection status.

Characteristics	Infection status		
	Symptomatic	Asymptomatic	Uninfected
No. of subjects	47	44	55
Men	21 (45)	16 (36)	35 (64)
Women	26 (55)	28 (64)	20 (36)
No. HSV-1 seropositive	19 (40)	22 (50)	0 (0)
Men	10 (21)	5 (11)	0 (0)
Women	9 (19)	17 (39)	0 (0)
Mean age (years)	39.0	38.5	37.4
Men	42.0	38.3	38.6
Women	36.5	38.6	35.3
Mean lesional recurrences/year	8.4	0	0
Men	8.8	0	0
Women	8.1	0	0

NOTE. Data in parentheses are % of subjects. Symptomatic and asymptomatic subjects were seropositive for HSV-2 and seronegative or seropositive for HSV-1; uninfected subjects were seronegative for both HSV-1 and -2.

Table 2. Gene frequencies at the human leukocyte antigen A (HLA-A) locus for subjects in 3 study cohorts.

	Symptomatic subjects (n = 47)			Asymptomatic subjects (n = 44)			Uninfected subjects (n = 55)		
HLA-A allele	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]
1	16	2	.191 ± .041	7	0	.080 ± .029	18	2	.182 ± .037
2	21	2	.245 ± .045	20	2	.249 ± .047	24	2	.236 ± .041
3	14	1	.160 ± .038	13	4	.190 ± .042	13	0	.118 ± .031
11	8	0	.085 ± .029	5	0	.057 ± .025	2	0	.018 ± .013
23	0	0	—	1	0	.011 ± .011	2	0	.018 ± .013
24	5	0	.053 ± .023	6	0	.068 ± .027	13	0	.118 ± .031
25	2	0	.021 ± .015	1	0	.011 ± .011	1	0	.009 ± .009
26	5	0	.053 ± .023	4	0	.045 ± .022	10	0	.091 ± .027
28	3	0	.032 ± .018	3	1	.042 ± .022	5	0	.045 ± .020
29	4	0	.043 ± .021	4	0	.045 ± .022	4	0	.036 ± .018
30	4	1	.053 ± .023	2	0	.023 ± .016	1	0	.009 ± .009
31	3	0	.032 ± .018	6	0	.068 ± .027	4	0	.036 ± .018
32	0	0	—	5	0	.057 ± .025	5	0	.045 ± .020
33	2	0	.021 ± .015	4	0	.045 ± .022	2	0	.018 ± .013
34	1	0	.011 ± .011	0	0	—	1	0	.009 ± .009
66	0	0	—	0	0	—	1	0	.009 ± .009
Blank		0	.000 ± .020		0	.008 ± .023		0	.000 ± .017
No. of codominant alleles		13			14			16	

[a] Antigen frequency.
[b] No. of subjects reacting to single antigen only.
[c] Estimated gene frequency ± SE.

uals. At the HLA-Cw locus, HLA-Cw7 is most common in all cohorts.

Among class II alleles, DR-13 is most frequently seen in participants with symptomatic HSV infection, and DR-4 is most frequent in individuals with asymptomatic HSV infection. In the uninfected population, both DR-13 and DR-4 are equally frequent isoforms. At the DQ loci, the DQ-2, -3, -5, and -6 isoforms are all prevalent.

Increased frequencies of individual genes in HSV-2–seropositive subjects. We compared the gene frequencies of the 91 HSV-2–infected symptomatic and asymptomatic subjects with those of the 55 HSV-seronegative subjects. The frequencies of several genes were significantly higher in HSV-2–seropositive subjects (table 7).

An increase of allele A11 frequency is borderline significant (P = .05). Significant elevations of frequencies of alleles B35 (P = .02) and B38 (P = .02) were found in infected subjects. These three allelic frequencies were not significant when adjustments for multiple comparisons were made. None of the class II (DR and DQ loci) genes were associated with HSV-2 infection.

The number of HSV-2–infected subjects with the Cw4 antigen was >3-fold greater than that among uninfected controls (27/91 = 0.30 vs. 5/55 = 0.09; odds ratio, 4.22). As shown in table 7, the increased Cw4 frequency was highly significant (P = .003). Even with application of Bonferroni's correction for multiple comparisons, the difference between infected and uninfected subjects was still significant (P = .027).

Differing gene frequencies among symptomatic and asymptomatic subjects with HSV-2 infection. Table 7 compares the estimated individual gene frequencies that had statistically significant differences between cohorts of symptomatically and asymptomatically infected subjects. HLA-A1, -DR13, and -DQ6 are positively associated with having frequent symptomatic recurrences of genital herpes. HLA-A32, -B27, and -Cw2, however, are negatively associated with symptomatic disease. Given the many alleles studied, particularly at the A and B loci, one may say that some comparisons would appear significant by chance. With statistical correction for multiple comparisons, however, the comparisons involving B27 and Cw2 still yielded strong trends toward significance (P = .08 and P = .06, respectively).

Frequencies of individual haplotypes in the study cohorts. The "autoimmune" haplotype, A1, B8, and DR3, occurred in 5 (10.6%) of 47 symptomatic patients, 2 (4.5%) of 44 asymptomatic patients, and 9 (16.4%) of 55 uninfected persons. Thus, the allelic frequency of these haplotypes in symptomatic persons was more than twice that in asymptomatic persons, but the difference did not achieve statistical significance. Moreover, the frequency (7.7%) of this haplotype among all 91 infected subjects was less than half that for uninfected subjects (P = .09). Of the 91 infected subjects, 24 (26.4%) had two antigens at different loci, B35 and Cw4, while 5 (9.1%) of 55 uninfected subjects had the two antigens at different loci. Fisher's exact test showed a significant association of the haplotype B35-Cw4 with infection (P = .01).

The frequencies of the haplotype A11, B35 also showed interesting differences among the study cohorts. This haplotype was seen in 4 (8.5%) of 47 symptomatic patients and in 2 (4.5%) of 44 asymptomatic patients but in no uninfected subjects. A positive association of A11, B35 with HSV-2 infection is almost significant using a one-tailed exact test (P = .055).

Table 3. Gene frequencies at the human leukocyte antigen B (HLA-B) locus for subjects in 3 study cohorts.

	Symptomatic subjects (n = 47)			Asymptomatic subjects (n = 44)			Uninfected subjects (n = 55)		
HLA-B allele	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]
7	9	0	.097 ± .030	10	0	.114 ± .034	15	3	.159 ± .035
8	10	0	.160 ± .032	4	0	.045 ± .022	11	1	.107 ± .030
13	7	0	.074 ± .027	4	0	.045 ± .022	2	0	.018 ± .013
14	4	0	.043 ± .021	5	0	.057 ± .025	7	0	.064 ± .023
18	2	0	.021 ± .015	4	0	.045 ± .022	7	0	.064 ± .023
27	0	0	—	7	0	.080 ± .029	9	0	.082 ± .026
35	14	0	.149 ± .037	14	1	.169 ± .040	6	0	.055 ± .022
37	1	0	.011 ± .011	0	0	—	0	0	—
38	3	0	.032 ± .018	3	0	.034 ± .018	0	0	—
39	0	0	—	1	0	.011 ± .011	1	0	.009 ± .009
41	3	0	.032 ± .018	1	0	.011 ± .011	1	0	.009 ± .009
44	12	0	.128 ± .034	10	2	.133 ± .036	12	1	.116 ± .031
45	1	0	.011 ± .011	0	0	—	1	0	.009 ± .009
47	0	0	—	1	0	.011 ± .011	0	0	—
49	0	0	—	2	0	.023 ± .016	2	0	.018 ± .013
50	1	0	.011 ± .011	2	0	.023 ± .016	0	0	—
51	7	0	.074 ± .027	1	1	.016 ± .013	5	0	.045 ± .020
52	1	0	.011 ± .011	0	0	—	1	1	.012 ± .010
53	1	0	.011 ± .011	2	0	.023 ± .016	0	0	—
55	0	0	—	1	0	.011 ± .011	1	0	.009 ± .009
56	0	0	—	0	0	—	1	0	.009 ± .009
57	2	0	.021 ± .015	2	0	.023 ± .016	3	0	.027 ± .016
58	4	0	.043 ± .021	0	0	—	1	0	.009 ± .009
60	7	0	.074 ± .027	3	0	.034 ± .019	10	0	.091 ± .028
61	0	0	—	1	0	.011 ± .011	2	0	.018 ± .013
62	4	0	.043 ± .021	4	0	.045 ± .022	5	0	.045 ± .020
63	1	0	.011 ± .011	1	0	.011 ± .011	0	0	—
70	0	0	—	1	0	.011 ± .011	1	0	.009 ± .009
Blank		0	.000 ± .016		0	.011 ± .021		0	.014 ± .020
No. of codominant alleles		20			23			22	

[a] Antigen frequency.
[b] No. of subjects reacting to single antigen only.
[c] Estimated gene frequency ± SE.

Discussion

Herein, we report MHC class I and class II antigen frequencies that are associated with HSV-2 infection or with the likelihood that HSV infection will be manifested by frequent symptomatic recurrences. These observations are compatible with known associations between polymorphisms at HLA loci and responses to other microbial pathogens, and they implicate host immune factors as determinants of the risks of HSV-2 infection and disease.

Coexpression of HSV peptides in the context of MHC class I and class II antigens is required for $CD8^+$ T cell killing of infected cells and for $CD4^+$ T cell–mediated regulation of B cell responses to viral proteins, respectively [29–31]. All herpes viruses studied possess mechanisms for deterring these host responses by down-regulating the presentation of their antigens together with MHC proteins and by severely limiting their antigen expression during latency. Epstein-Barr virus nuclear antigen-1 (EBNA-1), for example, is the only protein required for viral persistence in B cells, and it contains a glycine-alanine repeat motif that blocks proteosomal degradation and antigen presentation [32]. Cytomegalovirus encodes 4 gene products that, together, block multiple steps in MHC presentation [33, 34]. HSV-1– and -2–infected cell protein 47 (ICP47) blocks peptide transport into the Golgi complex, where ICP47 would normally bind to class I α chains, while the gene 41 protein shuts off host cell protein synthesis, including the MHC proteins [35, 36]. Further, HSV-associated fetal loss may be due to impaired trophoblast expression of HLA-G, a nonclassical class I protein with significant homology to HLA-A and -B, and resultant NK cell–mediated cytotoxicity [37, 38]. Moreover, HSV-1 and -2 persist in neurons, which normally display no class I molecules, and while latent, these express only one family of transcripts, which are not known to encode proteins [39]. For much of their residence in humans, then, HSV-1 and -2 are essentially invisible to the immune system. Immune responses to HSV are engaged only during the typically brief periods in which the virus is replicating in peripheral tissues while en route to or from its neuronal sanctuary. The nature and efficiency of these responses determine the outcome of each episode of infection [40].

The HLA isotype and the particular viral peptides that it binds are one set of factors that influence the outcome of a given herpesvirus infection. That the responsiveness of T cells from healthy seropositive individuals to HSV or cytomegalovirus glycoproteins B relates to the HLA haplotype provides

Table 4. Gene frequencies at the human leukocyte antigen Cw (HLA-Cw) locus for subjects in 3 study cohorts.

	Symptomatic subjects (n = 47)			Asymptomatic subjects (n = 44)			Uninfected subjects (n = 55)		
HLA-Cw allele	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]
1	5	2	.056 ± .024	2	1	.023 ± .016	6	1	.056 ± .022
2	0	0	—	6	1	.070 ± .028	6	3	.059 ± .023
3	12	3	.135 ± .036	8	0	.091 ± .031	19	4	.187 ± .039
4	13	4	.149 ± .038	14	7	.184 ± .043	5	2	.048 ± .021
5	6	1	.065 ± .026	4	2	.048 ± .023	10	2	.096 ± .029
6	12	6	.143 ± .037	8	0	.091 ± .031	6	0	.055 ± .022
7	18	5	.209 ± .044	20	7	.259 ± .050	29	9	.307 ± .047
8	2	1	.022 ± .015	3	1	.035 ± .020	6	0	.055 ± .022
Blank		2	.222 ± .049		2	.199 ± .050		1	.137 ± .041
No. of codominant alleles		7			8			8	

[a] Antigen frequency.
[b] No. of subjects reacting to single antigen only.
[c] Estimated gene frequency ± SE.

one indication that the MHC may modulate the pathogenesis of herpesvirus diseases [41, 42]. Moreover, particular MHC class I proteins determine which viral peptides can serve as targets for CTL killing. For example, EBNAs 3A, 3B, and 3C serve as CTL targets over a wide range of HLA backgrounds; however, responses to Epstein-Barr virus latent membrane protein 2 and EBNA 2 engage CTL with a limited number of HLA determinants [43].

Several epidemiologic studies addressed the clinical correlates of HLA alleles in HSV disease. Studies of a Sicilian population identified a negative correlation of HLA-B35 and a positive correlation of HLA-DR2 [17, 18] with HSV-1 infection, findings that were not confirmed for HSV-2 in our study. HLA associations with labial herpes infection in an Iraqi population showed significantly higher frequencies of HLA-A1, -B8, and -DR1 in the infected cohort; however, subgroup analyses found no associations with higher recurrence rates [19]. The high prevalence of HSV-1 infection worldwide, however, would imply that most of the HSV-1–negative controls in the Iraqi study were actually asymptomatic and not identifiable as such by the methods employed. If that is true, their higher frequency of A1 antigens would agree with the present data that distinguish symptomatic and asymptomatic persons with HSV-2 infection. The study of the Framingham cohort (1977–1979) found a decreased frequency of HLA-Bw16 and an increased frequency of Cw2 in individuals with a history of herpes labialis [20]. In that study, however, 63% of the control population relating no history of cold sores had detectable HSV-1 titers compared with 93% of the cohort giving a positive history, verifying the unreliability of patient history as a marker of serologic status.

Correlations between selected HLA allelic isoforms and postherpetic erythema multiforme have also been described and illustrate the potential impact of HLA and disease expression in herpes simplex infections. HLA-B62 and -B35 were significantly more frequent in affected patients, while the autoimmune haplotype A1, B8, and DR3 appeared to be protective [44]. A variety of studies showed that MHC class II isoforms HLA-DR1, -DR4, and -DR53 occurred more frequently in erythema multiforme–affected individuals; however, none of the studies confirmed the findings of the other [44–47].

Thus, there are common inconsistencies among studies of HLA associations with HSV infection. Some of the inconsistencies may have arisen because of multiple comparisons among data obtained from small study cohorts—a potentially valid concern regarding the present data, although we did perform statistical corrections to accommodate the multiple comparisons. In all of the prior HSV studies, however, an equally, if not more, important factor affecting their validity was the inability to accurately define the serologic status and to verify the clinical presentation of the subjects.

Table 5. Gene frequencies at the human leukocyte antigen DQ (HLA-DQ) locus for subjects in 3 study cohorts.

	Symptomatic subjects (n = 47)			Asymptomatic subjects (n = 44)			Uninfected subjects (n = 55)		
HLA-DQB1 allele	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]
02	18	1	.202 ± .042	19	3	.250 ± .047	20	2	.200 ± .039
03	22	3	.266 ± .046	24	5	.330 ± .051	29	5	.309 ± .045
04	5	0	.053 ± .023	3	0	.034 ± .019	3	0	.027 ± .016
05	16	1	.181 ± .040	16	2	.205 ± .043	19	1	.182 ± .037
06	25	3	.298 ± .048	14	1	.170 ± .040	29	2	.282 ± .044
Blank		0	.000 ± .033		0	.000 ± .031		0	.000 ± .030
No. of codominant alleles		5			6			5	

[a] Antigen frequency.
[b] No. of subjects reacting to single antigen only.
[c] Estimated gene frequency ± SE.

Table 6. Gene frequencies at the human leukocyte antigen DR (HLA-DR) locus for subjects in 3 study cohorts.

	Symptomatic subjects (n = 47)			Asymptomatic subjects (n = 44)			Uninfected subjects (n = 55)		
HLA-DR allele	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]	G[a]	No.[b]	f[c]
1	12	1	.138 ± .036	10	1	.123 ± .035	17	0	.155 ± .035
3	10	0	.106 ± .032	7	1	.088 ± .030	12	1	.118 ± .031
4	11	2	.138 ± .036	17	3	.223 ± .045	16	2	.164 ± .035
7	10	0	.106 ± .032	14	2	.178 ± .041	11	0	.100 ± .029
8	3	0	.032 ± .018	1	0	.011 ± .011	3	0	.027 ± .016
10	0	0	—	1	0	.011 ± .011	1	0	.009 ± .009
11	9	0	.095 ± .030	6	0	.068 ± .027	9	0	.082 ± .026
12	3	0	.032 ± .018	1	0	.011 ± .011	2	0	.018 ± .013
13	14	4	.191 ± .041	7	1	.088 ± .030	18	0	.164 ± .035
14	2	0	.021 ± .015	4	0	.045 ± .022	3	0	.027 ± .016
15	12	0	.128 ± .035	9	0	.102 ± .033	14	1	.136 ± .033
16	1	0	.011 ± .011	3	0	.034 ± .019	0	0	—
Blank		0	.000 ± .022		0	.016 ± .028		0	.000 ± .020
No. of codominant alleles		11			12			11	

[a] Antigen frequency.
[b] No. of subjects reacting to single antigen only.
[c] Estimated gene frequency ± SE.

In the present study, we used the Western blot assay to verify serologic status [10], and patients were documented clinically to have frequently recurring symptomatic genital herpes. A cohort of truly HSV-seronegative and, thus, uninfected subjects was established, and their HLA frequencies were compared with those of well-characterized HSV-2–infected cohorts. In so doing, we found several HLA loci to be associated with HSV-2 infection. Of these, the greatest proportional difference was with HLA-Cw4, which was detected in significantly more infected than uninfected subjects. Correlation of HLA-Cw4 with HSV infection may be due to inefficient or loss of appropriate triggering of the CTL response. Clearance of HSV-2 from genital lesions is associated with high-level CTL response [48]. The levels of HSV-specific $CD8^+$ CTL precursors are lower in HIV-infected patients who experience recurrent HSV-2 disease than in patients with mild HSV disease [49]. Further, HSV-1–infected cells are resistant to CTL-induced apoptosis [50]. Possibly, early and aggressive elimination of virally infected cells by CTL prevents HSV disease. That infection could be abrogated entirely is suggested by studies of HIV disease, in which innate immunity, as defined by chemokine receptor polymorphisms, does prevent HIV infection [51]. Proof that innate immunity could prevent HSV-2 infection would require detailed observations and daily cultures for virus shedding in well-characterized, serodiscordant couples.

HLA-B27 and HLA-Cw2 are associated with asymptomatic infection. In African-Americans, HLA-Cw2 is associated with an increased risk of developing multiple myeloma [52], while HLA-B27 is a well recognized risk factor for spondyloarthropathies and acute anterior uveitis [14]. HLA-B27–linked spondyloarthropathies arise following certain bacterial genitourinary infections [14]. Similarly, male HLA-B27/human β_2-microglobulin–transgenic mice develop spontaneous arthritis of the hind legs and nail changes when moved from sterile to conventional housing [53]. These mice are also susceptible to *Listeria monocytogenes,* developing severe inflammatory bowel disease and dying upon exposure [54]. Further, transfected human monocytic lines expressing HLA-B27 show impaired elimination of *Salmonella enteritidis* [55].

The cumulative data argue that MHC-linked factors contribute to disease symptomatology, although the nexus of immune interactions with HSV may be too complex to propose straightforward and traditional associations between HLA allelic frequencies and clinical outcomes. While HLA-B27,

Table 7. Comparison of estimated individual gene frequencies with significant differences between study cohorts.

	Infected (n = 91) vs. uninfected (n = 55)				Symptomatic (n = 47) vs. asymptomatic (n = 44)		
Allele	Difference (%) ± SE	χ^2	P	Allele	Difference (%) ± SE	χ^2	P
A11	5.3 ± 2.7	3.85	.05	A1	11.1 ± 5.0	4.92	.03
				A32	−5.7 ± 2.4	5.63	.02
B35	8.8 ± 3.7	5.66	.02	B27	−8.0 ± 2.8	8.15	.004
B38	4.4 ± 1.9	5.36	.02				
Cw4	11.7 ± 4.1	8.14	.003	Cw2	−7.0 ± 2.6	7.24	.007
				DR13	10.3 ± 5.2	3.92	.05
				DQB1∗06	12.8 ± 6.4	4.00	.05

which is often associated with autoimmunity, is correlated with asymptomatic genital herpes, the autoimmune haplotype, HLA-A1-B8-DR3, was observed twice as frequently in symptomatic than asymptomatic patients. Carriers of the B8-DR3 haplotype have a predominant Th2 profile [56]. Moreover, the HLA haplotype A1, B8, DR3 itself is a risk factor for HIV-related disease [16] and progression to AIDS [57]. HLA haplotype B8-DR3 also confers susceptibility to autoimmune-related hepatitis C virus mixed cryoglobulinemia [58]. This suggests that symptomatic infection has an immunopathologic component. Recent data on asymptomatic shedding are in accord with this possibility in that they demonstrate that fairly comparable quantities of infectious virus and viral DNA are recovered during symptomatic and asymptomatic outbreaks [59]. Thus, what appears to distinguish the episodes may be more the extent of host response–mediated injury than virus-inflicted injury.

The importance of NK cell activity in response to herpesvirus infections is exemplified by a patient who completely lacked an NK cell population. The patient developed severe varicella, cytomegalovirus, and herpes simplex infections in succession [60]. NK cell–mediated cytotoxicity presents a first-line antimicrobial response and is limited by NK cell inhibitory receptor recognition of HLA peptides. Group 1 and group 2 NK cells recognize HLA-Cw alleles; however, HLA binding of exogenous peptides is required to mediate the inhibitory signal [61–63]. of interest, loading of specific coxsackievirus peptide sequences or the superantigen glutamic acid decarboxylase onto HLA-Cw7 abrogates the normal inhibitory response of NK cells and may be causal in the development of autoimmune diabetes melitis [64]. The apparent protective role of HLA-Cw2 in HSV symptomatic disease may underlie a more robust initial immune response by NK cells, resulting in a diminished population of latent virus. Conversely, HLA modulation of the NK response may portend the vigor of the immune response to HSV reactivation.

How and whether the MHC alleles are themselves responsible for defining the variable host responses to HSV is unclear. The MHC itself appears to regulate cytokine production [56], presenting the possibility that elaborated cytokines may contribute to the risk of acquiring herpes infection and disease. Moreover, it is possible that other genes closely linked to and that cosegregate in populations with them are responsible [12, 65]. Various complement genes, tumor necrosis factor (TNF) genes, genes for peptide transporters, and the proteosome complex all map close to the MHC loci. In this regard, it is noteworthy that peptide transporter and TNF gene variants have been implicated in susceptibility to HIV and cerebral malaria (reviewed in [66]).

The present study suggests that purification of HSV peptides that bind particular HLA motifs associated with protection against infection or disease would provide rational components of immunoprophylactic or immunotherapeutic vaccines for genital herpes [67].

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References

1. Stanberry LR, ed. Genital and neonatal herpes. New York: John Wiley & Sons, **1996**.
2. Corey L, Spear PG. Infections with herpes simplex viruses. N Engl J Med **1986**; 314:686–91;749–57.
3. Koutsky LA, Stevens CE, Holmes KK, et al. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. N Engl J Med **1992**; 326:1533–9.
4. Mertz GJ, Schmidt O, Jourden JL, et al. Frequency of acquisition of first episode genital infection with herpes simplex virus from symptomatic and asymptomatic source contacts. Sex Transm Dis **1985**; 12:33–9.
5. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. Ann Intern Med **1996**; 124:8–15.
6. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. N Engl J Med **1995**; 333:770–5.
7. Rooney JJ, Felser JM, Ostrove JM, Straus SE. Acquisition of genital herpes from an asymptomatic sexual partner. N Engl J Med **1986**; 314:1561–4.
8. Brown ZA, Benedetti J, Ashley R, et al. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. N Engl J Med **1991**; 324:1247–52.
9. Mertz GJ, Benedetti J, Ashley R, Selke S, Corey L. Risk factors for sexual transmission of genital herpes. Ann Intern Med **1992**; 116:197–202.
10. Ashley RL, Militoni J, Lee F, et al. Comparison of Western blot (immunoblot) and G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol **1988**; 26: 662–7.
11. Rammensee HG, Bachmann J, Stevanovic S, eds. MHC ligands and peptide motifs. Molecular biology intelligence unit, Landes Bioscience. Austin, TX: Chapman & Hall, **1997**.
12. Erlich HA, McDevitt HO, Lock CB. The role of the MHC in autoimmune disease. In: Bona CA, Siminovitch KA, Zanetti M, Theofilopoulos AN, eds. The molecular pathology of autoimmune diseases. Chur, Switzerland: Harwood Academic Publishers, **1993**; 101–11.
13. Tiwari JL, Terasaki PI. HLA and disease associations. New York: Springer-Verlag, **1985**.
14. Lopez-Larrea C, Gonzalez-Roces S, Alverez V. HLA-B27 structure, function and disease association. Curr Opin Rheumatol **1996**; 8:296–308.
15. Strettell MD, Thomson LJ, Donaldson PT, Bunce M, O'Neill CM, Williams R. HLA-C genes and susceptibility to type 1 autoimmune hepatitis. Hepatology **1997**; 26:1023–6.
16. Steel CM, Ludlam CA, Beatson D, et al. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related diseases. Lancet **1988**; 1:1185–8.
17. Gallina G, Cumbo V, Messina P, Caprera V, Lio D, Caruso C. Lack of correlation between HLA-B35 resistance against herpes labialis and antibody titers to HSV-1. Oral Surg Oral Med Oral Pathol **1989**; 68:167–70.
18. Lio D, Caccamo N, d'Anna C, Cigna D, Candore G, Caruso C. Viral antibody titers are influenced by HLA-CD2 phenotype. Exp Clin Immunogenet **1994**; 11:182–6.

19. Jabbar AA, al-Samaria SA, al-Amar NS. HLA antigens associated with susceptibility to herpes simplex virus infection. Dis Markers **1991**;9:281–7.
20. Blackwelder WC, Dolin R, Mittal KK, McNamara PM, Payne FJ. A population study of herpesvirus infections and HLA antigens. Am J Epidemiol **1982**;115:569–76.
21. Terasaki PI. Histocompatibility testing. In: Danovitch GM, ed. Handbook of kidney transplantation. Boston: Little, Brown, **1991**;43–66.
22. Vartdal F, Gaudernack G, Funderud S. HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation, a fast and reliable technique. Tissue Antigens **1986**;28:301–12.
23. Park MS, Tonai RJ. Phenotypic frequencies of the class II (DR, DQ) DNA alleles by the patterns of sequence-specific primer mixtures (SSPM) in four different populations and the probable haplotypes between DRB1 allele and DQB1 allele. In: Terasaki PI, ed. Clinical transplants, 1992. Los Angeles: UCLA Tissue Typing Laboratory, **1993**;475–500.
24. Tonai RT, Terasaki PI. DNA typing of HLA-DR by sequence-specific primers. In: Nikaein A, ed. ASHI manual, 3d ed. **1994**;IV.C.4.1.
25. Nam J, Gart JJ. Berstein's and gene-counting methods in generalized ABO-like systems. Ann Hum Genet **1976**;39:361–73.
26. Gart JJ, Nam J. Statistical methods for genetic studies of HLA and cancer. In: Cornell RG, ed. Statistical methods in cancer studies. New York: Dekker, **1984**;229–66.
27. Armitage P. Statistical methods in medical research. New York: John Wiley & Sons, **1971**;131–8.
28. Miller RG Jr. Simultaneous statistical inference. New York: McGraw-Hill, **1966**.
29. Yasukawa M, Zarling JM. Human cytotoxic T cell clones directed against herpes simplex virus–infected cells. I. Lysis restricted by HLA class II MB and DR antigens. J Immunol **1984**;133:422–7.
30. Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM, Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. J Clin Invest **1998**;101:1500–8.
31. Posavad CM, Koelle DM, Corey L. Tipping the scales of herpes simplex virus reactivation: the important responses are local. Nat Med **1998**;4:381–2.
32. Levitskaya J, Coram M, Levitsy V, et al. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. Nature **1995**;375:685–8.
33. Wiertz E, Hill A, Tortorello D, Ploegh H. Cytomegaloviruses use multiple mechanisms to elude the host immune response. Immunol Lett **1997**;57:213–6.
34. Farrell HE, Vally H. Lynch DM, et al. Inhibition of natural killer cells by a cytomegalovirus MCH class I homologue in vivo. Nature **1997**;386:510–4.
35. Hill A, Jugovic P, York I, et al. Herpes simplex virus turns off the TAP to evade host immunity. Nature **1995**;375:411–5.
36. Tigges MA, Leng S, Johnson DC, Burke RL. Human herpes simplex virus (HSV)–specific $CD8^+$ CTL clones recognize HSV-2–infected fibroblasts after treatment with IFN-γ or when virion host shutoff functions are disabled. J Immunol **1996**;156:3901–10.
37. Schust DJ, Hill AB, Ploegh HL. Herpes simplex virus blocks intracellular transport of HLA-G in placentally derived human cells. J Immunol **1996**;157:3375–80.
38. 'Pazmany L, Mandelboim O, Vales-Gomez M, Davis DM, Reyburn HT, Strominger JL. Protection from natural killer cell–mediated lysis by HLA-G expression on target cells. Science **1996**;274:792–4.
39. Wagner EK, Bloom DC. Experimental investigation of herpes simplex virus latency. Clin Microbiol Rev **1997**;10:419–43.
40. Posavad CM, Koelle DM, Shaughnessy MF, Corey L. Severe genital herpes infections in HIV-infected individuals with impaired herpes simplex virus–specific $CD8^+$ cytotoxic T lymphocyte responses. Proc Natl Acad Sci USA **1997**;94:10289–94.
41. Chan WL, Tizard ML, Faulkner L. Proliferative T-cell response to glycoprotein B of the human herpesviruses: the influence of MHC and sequence of infection on the pattern of cross-reactivity. Immunology **1989**;68:96–101.
42. Curtsinger JM, Liu YN, Radeke R, Bryon MK, Fuad S, Bach FH, Gehrz RC. Molecular analysis of the immune response to human cytomegalovirus glycoprotein B (gB). II. Low gB-specific T and B cell responses are associated with expression of certain HLA-DR alleles. J Gen Virol **1994**;75:301–7.
43. Murray RJ, Kurilla MG, Brooks JM, Thomas WA, Rowe M, Kieff E, Rickinson AB. Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. J Exp Med **1992**;176:157–66.
44. Schofield JK, Tatnall FM, Brown J, McCloskey D, Navarette C, Leigh IM. Recurrent erythema multiforme: tissue typing in a large series of patients. Br J Dermatol **1994**;131:532–5.
45. Khalil I, Lepage V, Douay C, et al. HLA DQB1∗0301 allele is involved in the susceptibility to erythema multiforme. J Invest Dermatol **1991**;97:697–700.
46. Simon M, Fuchs C. HLA pattern in patients with post-herpetic erythema exsudativum multiforme. Z Hautkr **1990**;65:303–4.
47. Kampgen E, Burg G, Wank R. Association of herpes simplex virus–induced erythema multiforme with the human leukocyte antigen DQw3. Arch Dermatol **1988**;124:1372–5.
48. Koelle DM, Posavad CM, Barnum GR, Johnson ML, Frank JM, Corey L. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. J Clin Invest **1998**;101:1500–8.
49. Jerome KR, Tait JF, Koelle DM, Corey L. Herpes simplex virus type 1 renders infected cells resistant to cytotoxic T-lymphocyte–induced apoptosis. J Virol **1998**;72:436–41.
50. Posavad CM, Koelle DM, Shaughnessy MF, Corey L. Severe genital herpes infection in HIV-infected individuals with impaired herpes simplex virus–specific $CD8^+$ cytotoxic T lymphocytes responses. Proc Natl Acad Sci USA **1997**;94:10289–94.
51. Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Science **1996**;273:1856–62.
52. Pottern LM, Gart JJ, Nam J, et al. HLA and multiple myeloma among black and white men: evidence of a genetic association. Cancer Epidemiol Biomarkers Prev **1992**;1:177–82.
53. Khare SD, Hansen J, Luthra HS, David CS. HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human β_2-microglobulin ($\beta 2_m$) double transgenic mice with disrupted mouse β_2m. J Clin Invest **1996**;98:2746–55.
54. Warner TF, Madsen J, Starling J, Wagner RD, Taurog JD, Balish E. Human HLA-B27 gene enhances susceptibility of rats to oral infection by *Listeria monocytogenes.* Am J Pathol **1996**;149:1737–43.
55. Laitio P, Virtala M, Salmi M, Pelliniemi LJ, Yu DT, Granfors K. HLA-B27 modulates intracellular survival of *Salmonella enteritidis* in human monocytic cells. Eur J Immunol **1997**;27:1331–8.
56. Caruso C, Candore G, Modica MA, et al. Major histocompatibility complex regulation of cytokine production. J Interferon Cytokine Res **1996**;16:983–8.
57. McNeil AJ, Yap PL, Gore SM, et al. Association of HLA types A1-B8-DR3 and B27 with rapid and slow progression of HIV disease. QJM **1996**;89:177–85.
58. Lenzi M, Frisoni M, Mantovani V, et al. Haplotype HLA-B8-DR3 confers susceptibility to hepatitis C virus–related mixed cryoglobulinemia. Blood **1998**;91:2062–6.
59. Wald A, Corey L, Cone R, Hobson A, Davis G, Zeh J. Frequent genital herpes simplex virus 2 shedding in immunocompetent women. Effect of acyclovir treatment. J Clin Invest **1997**;99:1092–7.

60. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med **1989**;320:1731–5.
61. Biassoni R, Falco M, Cambiaggi A, et al. Amino acid substitutions can influence the natural killer (NK)–mediated recognition of HLA-C molecules. Role of serine-77 and lysine-80 in the target cell protection from lysis mediated by "Group2" or "Group1" NK clones. J Exp Med **1995**; 182:605–9.
62. Zappacosta F, Borrego F, Brooks AG, Parker KC, Coligan JE. Peptides isolated from HLA-Cw∗0304 confer different degrees of protection from natural killer cell–mediated lysis. Proc Natl Acad Sci USA **1997**;94: 6313–8.
63. Rajagopalan S, Long EO. The direct binding of a p58 killer cell inhibitory receptor to human histocompatibility leukocyte antigen (HLA) Cw4 exhibits peptide selectivity. J Exp Med **1997**;185:1523–8.
64. Mandelboim O, Wilson SB, Vales-Gomez M, Reyburn HT, Strominger JL. Self and viral peptides can initiate lysis by autologous natural killer cells. Proc Natl Acad Sci USA **1997**;94:4604–9.
65. Merriman TR, Todd JA. Genetics of autoimmune disease. Curr Opin Immunol **1995**;7:786–92.
66. Hill AVS. HIV and HLA: confusion or complexity. Nat Med **1996**;2:395–6.
67. Davenport M, Hill AVS. Reverse immunogenetics: from HLA-disease associations to vaccine candidates. Mol Med Today **1996**;2:38–45.